Radiation-Induced Erythroleukemia in the Beagle Dog

A Hematologic Summary of Five Cases

David V. Tolle, MS, Thomas E. Fritz, DVM, and William P. Norris, PhD

Eleven cases of myeloproliferative disease occurred in a group of 24 beagle dogs placed in a ^{60}Co γ-ray field at about 13 months of age and irradiated at an exposure rate of 5 R/22-hour day for duration of life. Of these 11 dogs, 5 (described in this paper) were diagnosed as having erythroleukemia. The bone marrow showed marked erythroblastic hyperplasia, with maturation arrest of the erythroid elements, and increased numbers of myeloblasts and promyelocytes. The terminal peripheral blood was characterized by marked anemia and thrombocytopenia, with circulating erythrocytic precursors and abnormal erythrocyte morphology. Splenomegaly and hepatomegaly occurred in 4 of the 5 animals. In the spleens and livers of all 5, there was extensive leukemic infiltration and proliferation. The extent of leukemic involvement in other tissues and organs varied in individual dogs. (Am J Pathol 87:499–510, 1977)

ERYTHROLEUKEMIA is included among the myeloproliferative disorders by Dameshek.[1] It is a mixed cell type leukemia, in which there is concurrent proliferation of both erythroblasts and myeloblasts. The predominant cell, however, is the basophilic pronormoblast. This condition differs from both erythremic myelosis, in which normocytic erythroblasts predominate with little or no increase in myeloblasts, and from Di Guglielmo's syndrome,[2] in which the predominant cell is a megaloblastoid erythroblast which is often PAS positive.[3]

Erythroleukemia, erythremic myelosis, and Di Guglielmo's syndrome have been reported mainly in man.[4-11] Erythroleukemia is rare in domestic animals with the exception of the chicken,[12] in which the disease is known to be of viral origin.[13] Spontaneous erythroleukemia, erythroid leukosis, and erythremic myelosis have been reported in the cat,[14-16] dog,[17] and pig.[18] Only 2 cases of irradiation-induced erythroleukemia have been described in the dog.[19,20]

In this paper, we describe the progressive changes in the peripheral blood and the hemopathology of irradiation-induced erythroleukemia with the intent to define this malignancy as a potential model for the study of the human disease.

From the Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois.

Supported by the US Energy Research and Development Administration.

Address reprint requests to David V. Tolle, Division of Biological and Medical Research, Building 202, Argonne National Laboratory, 9700 South Cass Avenue, Argonne, IL 60439.

Materials and Methods

Thirteen-month-old purebred beagle dogs of both sexes were caged individually in a ^{60}Co γ-ray field and thereafter irradiated continuously, 22 hours/day, at 5 R/day until death. Complete hemograms were obtained from jugular blood samples at 14-day intervals for the duration of the experiment. Red (RBC) and white (WBC) blood cells were counted electronically. Hemoglobin (Hb) was measured spectrophotometrically as cyanmethemoglobin at 540 nm. The volume of packed red cells (VPRC) was determined by the microhematocrit method, and thrombocytes were counted with phase contrast optics.[21] Differential leukocyte counts of 200 cells were made from thin films of fresh peripheral blood stained with Wright's stain.

Immediately after death, or sacrifice of moribund animals, a complete necropsy was performed. Bone marrow touch imprints were made from the split femur. Imprints were also taken from the liver, spleen, and various lymph nodes to evaluate both the cytology and the degree of extramedullary hematopoiesis. Imprints were routinely stained with Wright's-Giemsa stain; in some cases, peroxidase,[22] Sudan black B,[23] and periodic acid–schiff (PAS) [24] stain was used for diagnostic confirmation of immature cells. Differential counts of 1000 bone marrow cells were made, and the myeloid-erythroid ratio (M/E) was calculated.

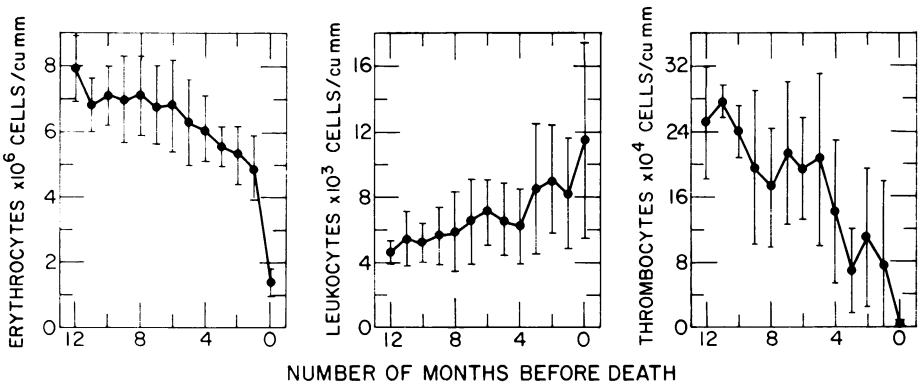
Results

Hematology

The progressive changes in the peripheral blood values of the 5 dogs with erythroleukemia described herein do not differ greatly from those changes previously described in 12 dogs with radiation-induced granulocytic leukemia seen at this laboratory.[20]

The mean (± SD) RBC, WBC, and thrombocyte numbers for the 5 animals during the 12-month period before death are shown in Text-figure 1. Representative hemograms at various times before death are given in Table 1.

Abnormal erythrocyte morphology increased with time and was most



TEXT-FIGURE 1—Mean (± SD) peripheral blood leukocyte, erythrocyte, and thrombocyte numbers during the 12-month period before death in 5 dogs dying with radiation-induced erythroleukemia.

Table 1—Peripheral Hematologic Data

Dog no.	Days before death	RBC $\times 10^6$/ cu mm	WBC*/ cu mm	Hb (g/100 ml)	VPRC (%)	Thrombocytes $\times 10^3$/ cu mm	NRBC/ 200 WBC
1392	250	7.79	8,537	18.8	57	255	10
	194	7.09	8,226	18.8	56	250	0
	138	7.17	10,460	20.0	58	270	6
	82	6.36	7,668	16.3	49	245	0
	26	6.48	6,623	17.2	52	57	8
	1	0.98	3,517	1.2	8	3	201
1439	89	6.12	8,773	15.8	42	140	0
	61	5.50	15,836	14.2	39	57	6
	33	4.87	14,032	11.4	34	23	1
	11	3.55	15,195	8.4	23	3	5
	5	2.78	17,905	6.0	18	1	4
	1	1.73	18,807	3.8	12	1	6
1469	112	6.67	5,292	16.6	46	152	0
	84	5.58	4,734	14.4	39	75	2
	57	5.28	5,315	13.2	37	52	0
	28	4.92	6,032	11.6	34	13	9
	14	4.58	6,402	10.8	31	5	82
	2	2.07	10,490	4.6	14	2	24
1453	78	5.53	6,663	14.4	43	132	92
	57	5.13	9,351	13.6	41	157	50
	38	4.54	8,591	11.8	36	260	40
	10	3.38	7,832	10.0	29	29	48
	4	1.86	5,745	4.6	15	12	6
	0	1.17	9,009	3.0	8	1	1
1366	203	5.02	9,936	12.4	37	110	2
	161	5.20	8,754	13.0	38	63	0
	77	4.18	7,489	10.2	32	23	5
	43	3.57	7,810	9.4	28	26	4
	7	2.65	7,416	6.4	21	3	77
	0	1.21	15,709	4.0	11	1	298

VPRC = volume of packed red cells, NRBC = nucleated red blood cells.
* Corrected for nucleated red blood cells: $A/(A + B) \times$ total WBC = NRBC corrected WBC. A = number of WBC identified, B = number of NRBC identified.

severe just before death, at which time all 5 dogs showed marked terminal anemia and thrombocytopenia.

Two-hundred to 400 days before death, various abnormalities in circulating erythrocytes were common (e.g., anisocytosis, poikilocytosis, polychromatophilic macrocytes, target cells, Howell-Jolly bodies, and nucleated red blood cells [NRBC]). Although some animals showed an increase in neutrophilic band cells and metamyelocytes during this period, circulating erythroblasts and myeloblasts were not observed either on peripheral blood films or in buffy coat preparations.

Thirty to 60 days before death, all dogs showed increased numbers of

immature granulocytes, including myelocytes, promyelocytes, and occasional myeloblasts. Circulating NRBC were present in all 5 dogs, and included erythroblasts in 3 dogs. Other abnormalities regularily observed 30 to 60 days before death were giant thrombocytes, cytoplasmic vacuolation, cytoplasmic blebs, giant neutrophilic bands, and hypersegmented mature neutrophils. Occasionally, erythroid precursors with bizarre multiple nuclei and abnormal mitotic figures were observed in the peripheral blood (Figures 1 and 2).

One animal (1453) consistently had 100 to 175 NRBC/200 WBC on peripheral blood films from 6 months to 3 months before death. During the last month the number of NRBC dropped dramatically.

The terminal differential leukocyte counts of peripheral blood (Table 2) show the degree of shift to immature granulocytes. None of the dogs had a terminal leukocytosis, the highest WBC count being 18,807/cu mm.

Pathology

At necropsy, splenomegaly and hepatomegaly were present in 4 of the 5 animals (Table 3), and lymphadenopathy was present in all.

Bone Marrow

The bone marrow of all dogs was hyperplastic, dark red, and pulpy, with little or no normal marrow fat. Touch imprints showed marked erythroid hyperplasia with reduction in the granulocytic and megakaryocytic elements and very little fat (Figures 3 and 4). Although erythroblastic proliferation and maturation arrest were the prominent microscopic features, the percentage of myeloblasts, promyelocytes, and

Table 2—Terminal Differential Leukocyte Counts

	Dog 1392	Dog 1439	Dog 1469	Dog 1453	Dog 1366
Mature neutrophils (%)	38.0	52.5	33.5	2.5	23.0
Bands (%)	35.0	22.0	36.0	43.0	38.5
Metamyelocytes (%)	10.5	10.5	20.0	29.0	18.5
Myelocytes (%)	2.5	6.5	3.0	17.5	4.5
Promyelocytes (%)	0.5	0.5	0.5	1.0	1.5
Myeoloblasts (%)	3.5	0.5	2.0	2.5	3.0
Lymphocytes (%)	10.0	7.0	5.0	2.5	10.0
Monocytes (%)	0.0	0.0	0.0	2.0	1.0
Eosinophils (%)	0.0	0.5	0.0	0.0	0.0
NRBC/200 WBC	201	6	24	1*	298
Total days irradiated	989	1430	1440	1803	1949

* Between 175 days and 90 days before death, a value of 100 to 175 NRBC/200 WBC was a constant finding on peripheral blood films from this dog. During the last 30 days before death the number of circulating NRBC declined rapidly.

Table 3—Spleen and Liver Weights

Dog No.	Spleen (g)	Liver (g)
1392	27.7	262.2
1439	195.2	449.3
1469	221.7	526.8
1453	262.7	370.9
1366	188.8	538.2
Control*	24.9 ± 1.2	323.2 ± 13.5

* Values are from 44 adult nonexperimental beagles.

myelocytes was also increased. The bone marrow differential cell counts and M/E ratios are given in Table 4. Increased numbers of plasma cells and macrophages laden with debris and showing erythrophagocytosis were common. In 2 cases of marked erythroblastic proliferation (Dogs 1439 and 1469), the erythroid precursors were negative for the PAS stain.

Spleen

At necropsy the spleen in all 5 animals was dark red and pulpy. Touch imprints showed prominent infiltration by and proliferation of, leukemic erythroblasts as well as myeloid precursors and plasma cells (Figure 5). In 4 of the dogs the cellular features of the spleen resembled those of the bone marrow; few normal lymphoid elements were present in either tissue.

Liver

In all dogs, there was significant hematopoiesis in the liver since the majority of cells were erythroblasts (Figure 6). The degree of liver involvement varied from focal infiltration and proliferation of hemato-

Table 4—Differential Cell Counts in Bone Marrow of Dogs Dying With Erythroleukemia

	Dog 1392	Dog 1439	Dog 1469	Dog 1453	Dog 1366	Controls* (mean ± SE)
Myeloblasts (%)	5.0	1.5	0.5	2.0	0.5	0.4 ± 0.1
Promyelocytes (%)	7.0	1.0	3.0	4.5	3.0	1.1 ± 0.2
Myelocytes (%)	11.0	4.0	6.5	26.5	5.0	10.6 ± 0.6
Metamyelocytes (%)	2.0	4.5	5.5	8.5	5.5	9.1 ± 0.5
Bands (%)	2.0	12.5	1.5	5.5	4.5	15.6 ± 0.8
Segmented cells (%)	0.0	9.0	0.0	1.5	0.5	20.3 ± 1.2
Rubriblasts (%)	13.0	22.5	32.5	10.0	18.5	0.2 ± 0.1
Prorubricytes (%)	13.0	10.0	23.0	11.0	12.0	2.9 ± 0.3
Rubricytes (%)	16.0	16.0	13.0	13.5	18.5	16.2 ± 0.3
Metarubricytes (%)	23.0	11.5	8.0	11.5	27.5	20.6 ± 0.8
1000 cell M/E ratio	0.3	0.6	0.2	1.0	0.2	1.5 ± 0.1

Values do not add to 100% because only granulocytic and erythrocytic cells are included in this table.

* Thirty-five clinically normal, untreated adult dogs.

poietic elements to massive infiltration severe enough to cause distortion of the normal architecture.

Lymph Nodes

Three or more lymph nodes from each dog were excised for imprints. In any given dog, the degree of leukemic involvement varied among individual nodes. Severely affected nodes had nearly complete replacement of the normal architecture by erythroblasts (Figure 7) and showed changes similar to those seen in the spleen. Less severely affected nodes had leukemic infiltration of cortical nodules, trabeculae, and medullary cords. In all lymph node imprints there were increased numbers of plasma cells.

Discussion

Because erythroleukemia and related myeloproliferative disorders similar to those seen in man are extremely rare in most domestic animals,[12,20] any experimentally induced case is worthy of note. In the present study, 11 cases of myeloproliferative disorder including the 5 of erythroleukemia described here in detail, developed in 24 beagle dogs receiving continuous whole-body exposure to ^{60}Co γ-radiation. The severity and rate at which anemia and thrombocytopenia developed varied among the dogs, but in 4 of the 5 animals the process was gradual (Table 1). In the fifth animal (No. 1392), the rapid decline in the RBC count (5.5×10^6 cells/cu mm within 1 month), probably reflects an acute phase of the leukemic process since clinical and necropsy findings did not indicate significant hemorrhage. The sharp reduction terminally in the percentage of circulating NRBC seen in another animal (No. 1453) has been noted in human cases of Di Guglielmo's syndrome during acute myeloblastic transition from the erytholeukemic phase.[2]

Terminal anemia and thrombocytopenia is common in erythroleukemia and has been termed *anerythroblastic anemia,* a condition in which anemia coexists with increased numbers of peripheral NRBC and a bone marrow crowded with erythroid precursors. This hematologic picture closely resembles the "refractory normoblastic anemias,"[2] except for the widespread leukemic infiltration of not only potential hematopoietic tissue but also of nonhematopoietic tissue as well (i.e., skin, kidney, lung, heart, and gastrointestinal wall).[20] This mixed erythroblastic-myeloblastic infiltration is an uncontrolled response which, by definition, is neoplastic and constitutes a leukemia that morphologically closely resembles erythroleukemia in man.

Since the finding of such a high incidence of myeloproliferative disorders in these chronically irradiated animals was not expected, serial

bone marrow biopsies were not included in the experimental protocol. New studies are now underway to attempt to define, by light and electron microscopy and *in vitro* culture techniques, the sequential marrow cellular and structural changes associated with the leukemogenic process and to evaluate further the potential usefulness of the canine system as a model for the study of the human disease.

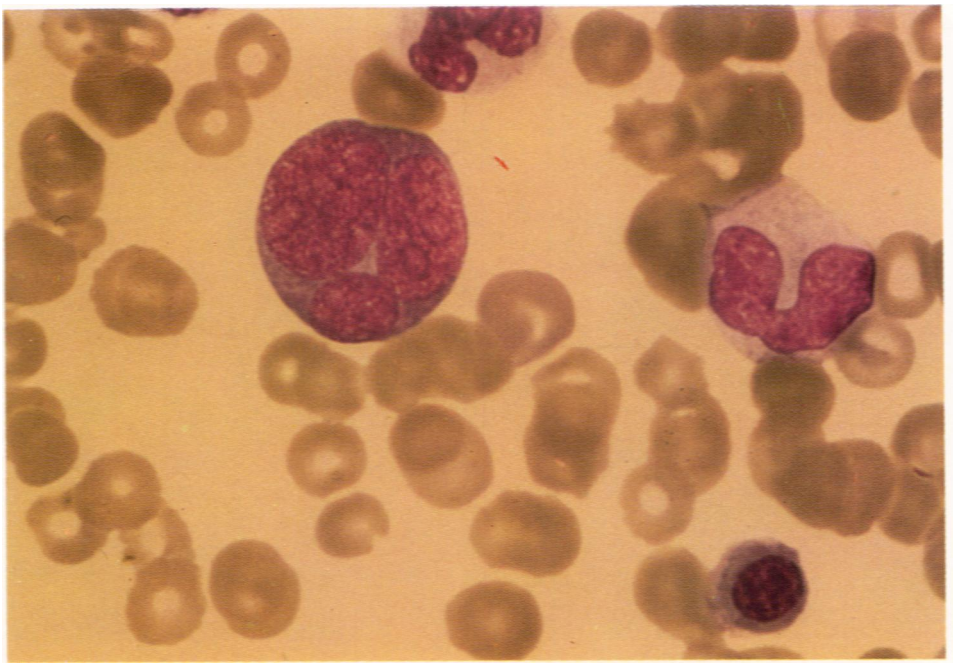
References

1. Dameshek W: Some speculations on the myeloproliferative syndromes. Blood 6:372–375, 1951
2. Dameshek W, Gunz F: Leukemia and the Myeloproliferative Disorders. Leukemia, Second edition. Edited by W Dameshek, F Gunz. New York, Grune and Stratton, 1964, pp 356–402
3. Quaglino D, Hayhoe FGJ: Periodic-acid-schiff positivity in erythroblasts with special reference to Di Guglielmo's disease. Br J Haematol 6:26–33, 1960
4. Cowles J, Saikkonen J, Thorell B: On the presence of hemoglobin in erythroleukemia cells. Blood 13:1176–1184, 1958
5. Di Guglielmo G, Ferrara A: Contributo alla diagnostica della malattie eritremiche ed eritroleucemiche. Haematologica 41:605–655, 1956
6. Finkel HE, Brauer MJ: Giant eosinophilic granules in the chronic Di Guglielmo syndrome: A clue to the diagnosis of myeloid neoplasia. N Engl J Med 274:209–210, 1966
7. Heath CW, Moloney WC: Cytogenetic observations in a case of erythremic myelosis. Cancer 18:1495–1504, 1965
8. Martin WJ, Bayrd ED: Erythroleukemia, with special emphasis on the acute or incomplete variety: Report of five cases. Blood 9:321–339, 1954
9. Moeschlin S: Erythroblastosen, erythroleukämien, und erythroblastämien. Folia Haematol 64:262–302, 1940
10. Sarvajic AJ, Komninos Z: "Pure" acute erythremic myelosis. NY State J Med 66:636–641, 1966
11. Schwartz SO, Critchlow J: Erythremic myelosis (Di Guglielmo's disease): Critical review with report on four cases, and comments on erythroleukemia. Blood 7:765–793, 1952
12. Moulten JE: Tumors In Domesticated Animals. Berkeley, University of California Press, 1961
13. Beard JW: Etiology of avian leukosis. Ann NY Acad Sci 68:473–486, 1957
14. Schalm OW, Jain NC, Carroll EJ: Veterinary Hematology, Third edition. Philadelphia, Lea and Febiger, 1975, pp 579–581
15. Storti E, Brotto M:. Über die Pathogenese der übertragboren Hühnerleukämie. Folia Haematol 63:1–33, 1939
16. Zawidzka ZZ, Janzen E, Grice HC: Erythremic myelosis in a cat: A case resembling Di Guglielmo's syndrome in man. Pathol Vet 1:530–541, 1964
17. Liu SK, Carb AV: Erythroblastic leukemia in a dog. J Am Vet Med Assoc 152:1511–1516, 1968
18. Englert HK: Die Leukose des Schweines. Zentralbl Veterinaermed 2:607–628, 764–801, 1955
19. Anderson AC, Johnson RM: Erythroblastic malignancy in a beagle. J Am Vet Med Assoc 141:944–946, 1962
20. Fritz TE, Norris WP, Tolle DV: Myelogenous leukemia and related myeloproliferative disorders in beagles continuously exposed to $^{60}Co\ \gamma$ -radiation. Unifying Concepts of Leukemia. Bibl Haematol 39:170–188, 1973

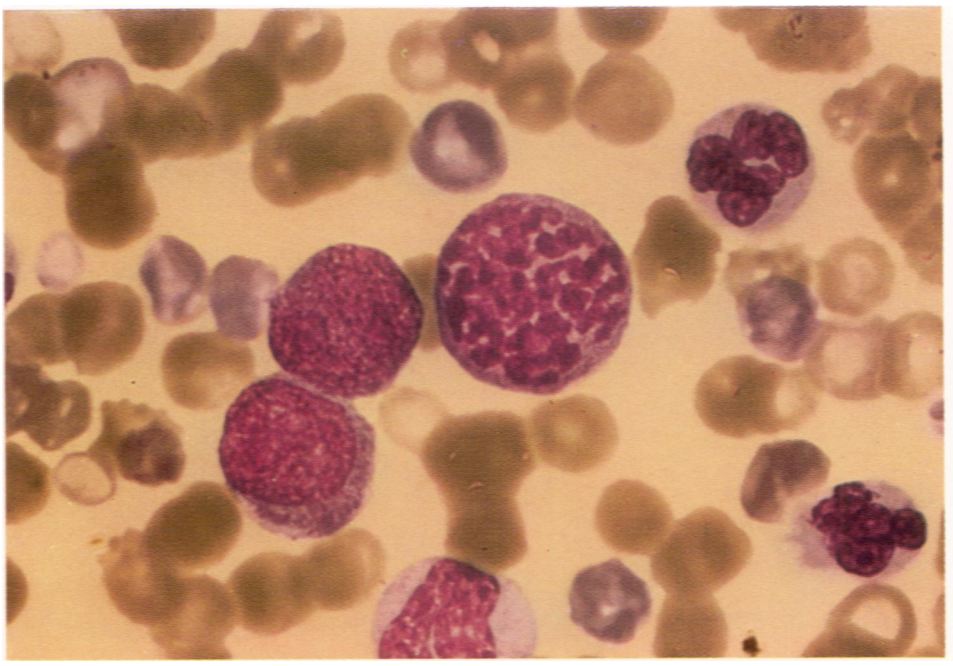
21. Brecher G, Cronkite EP: Morphology and enumeration of human blood platelets. J Appl Physiol 3:365–377, 1950
22. Sato A, Sekiya S: A simple method for differentiation of myeloic and lymphatic leukocytes of the human blood. Tohoku J Exp Med 7:111–115, 1926
23. Sheehan HL, Storey GW: An improved method of staining leucocyte granules with Sudan black B. J Pathol 59:336–337, 1947
24. Hayhoe FGJ, Flemans RJ: An Atlas of Haematological Cytology. New York, Wiley-Interscience, 1970, pp 316–317

Acknowledgments

The authors gratefully acknowledge the contributions of Donald Doyle, Donald Pearson (deceased), Patrick Polk, Susan Cullen, and the veterinary support and animal care staff during the course of this work.



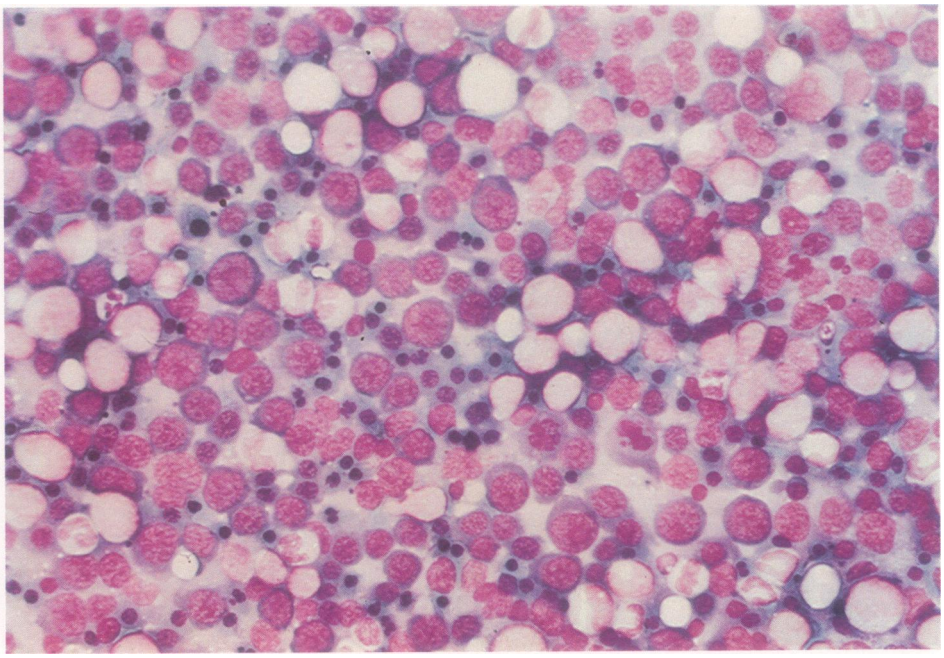
1



2

Figure 1—Peripheral blood film of Dog 1469 14 days before death showing a bizarre erythroblast with three nuclei and prominent nucleoli (Wright's-Giemsa stain, × 1715). **Figure 2**—Peripheral blood film of Dog 1469 2 days before death. A prometaphase stage erythroblast with abnormally heavy chromosomal condensation is shown. Hypochromatophilia, anisocytosis, and poikilocytosis are seen in the mature erythrocytes. (Wright's-Giemsa stain, × 1715).

3



4

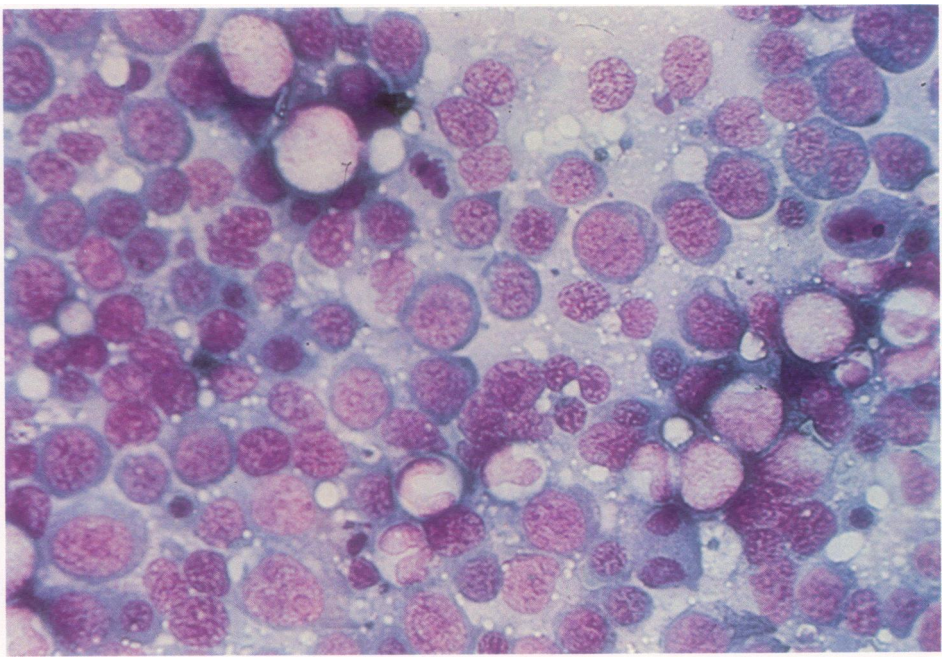
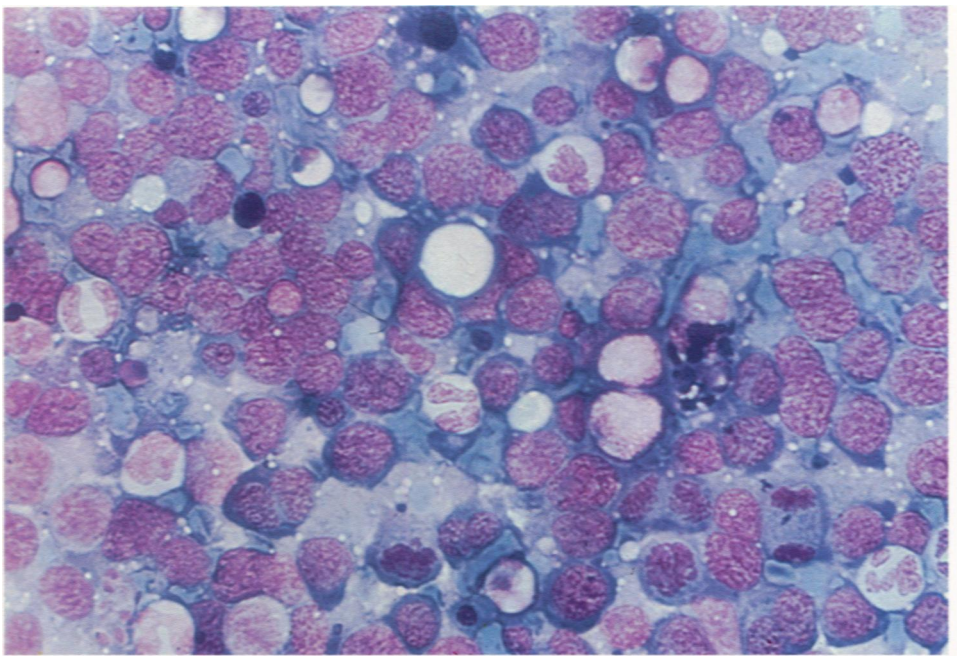
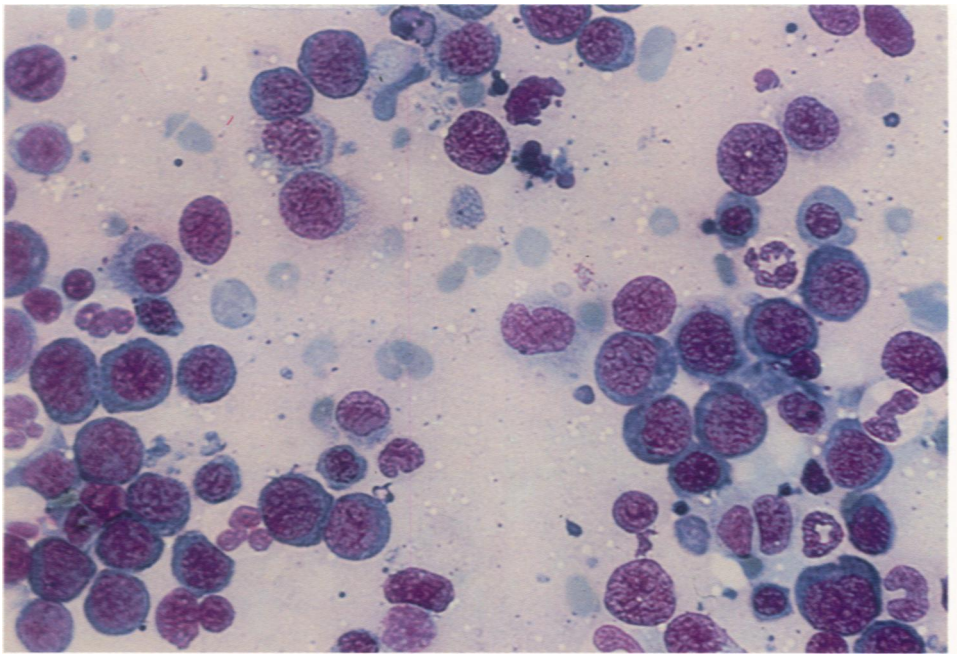


Figure 3—Terminal bone marrow impression from Dog 1366 showing erythroid hyperplasia with moderate maturation arrest. There is a reduction of myeloid elements and an absence of megakaryocytes. Note the reduction of the amount of normal fat. (Wright's-Giemsa stain, × 430). **Figure 4**—Terminal bone marrow impression from Dog 1469 showing marked erythroblastic proliferation and severe maturation arrest. Occasional mitotic figures and binucleate cells are seen. (Wright's-Giemsa stain, × 685)



5



6

Figure 5—Terminal spleen impression from Dog 1469 showing erythroblastic replacement of normal lymphocytic elements (Wright's-Giemsa stain, × 685). **Figure 6**—Terminal liver impression from Dog 1469, showing the majority of cells to be erythroblasts (Wright's-Giemsa stain, × 685).

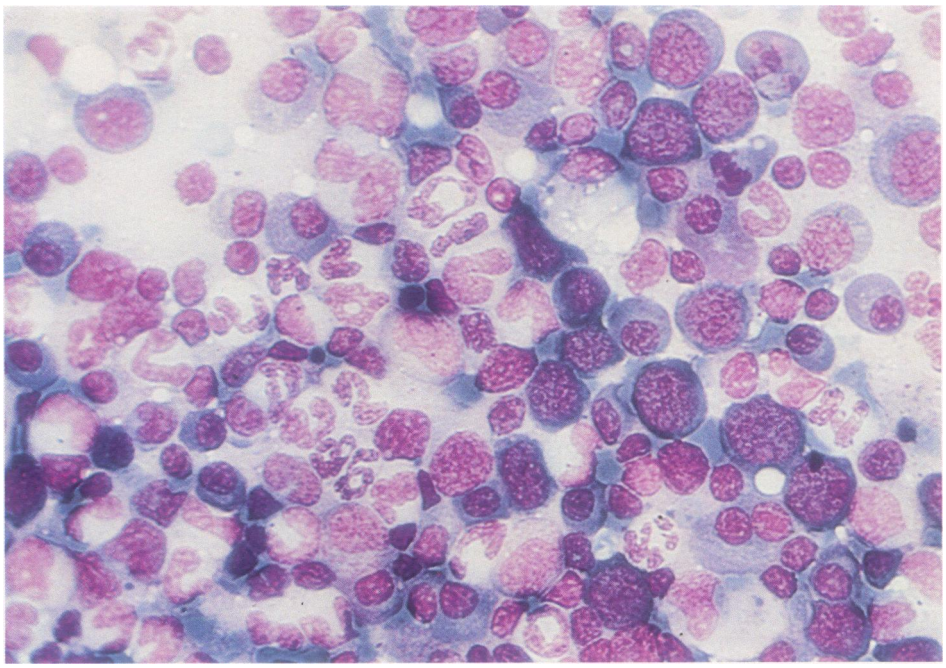


Figure 7—Terminal iliac lymph node impression from Dog 1439. The erythroid elements show maturation arrest while the myeloid elements show normal differentiation. There is an increase of plasma cells and an absence of lymphocytes. (Wright's-Giemsa stain, × 685)